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# (54) WTI MODIFIED PEPTIDE

(57) The present invention discloses a cancer antigen peptide comprising the following amino acid sequence: Cys Tyr Thr Trp Asn Gln Met Asn Leu (Sequence ID No. 3), a cancer vaccine having this for its active ingredient, and a DNA vaccine having for its active ingredient DNA that codes for this peptide.

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## Description

### Technical Field

**[0001]** The present invention relates to a cancer antigen based on the product of Wilms' tumor suppresser gene WT1. This cancer antigen is useful as an anticancer vaccine against cancers of the blood such as leukemia, myelodysplastic syndrome, multiple myeloma and malignant lymphoma, solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germinal cell cancer, liver cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical carcinoma and ovarian cancer, as well as any cancer that expresses WT1.

### Description of the Related Art

**[0002]** The immune mechanism for eliminating foreign objects from the body generally consists of humoral immunity, in which is involved macrophages that function as antigen-presenting cells that recognize an antigen, helper T-cells that activate other T-cells by recognizing antigens presented by said macrophages and producing various lymphokines, and B lymphocytes that differentiate into antibody-producing cells due to the action of said lymphokines; and, cellular immunity, by which killer T-cells (cytotoxic T-cells (CTL)), which have differentiated as a result of being presented with an antigen, attack and destroy target cells.

**[0003]** At present, cancer immunity is thought to mainly be the result of cellular immunity involving killer T-cells. In cancer immunity affected by killer T-cells, precursor T-cells, which have recognized cancer antigen presented in the form of a complex of major histocompatibility complex (MHC) class I (MHC class I antigen, also referred to as HLA antigen in the case of humans) and cancer antigen, differentiate and proliferate, and the resulting killer T-cells that have formed attack and destroy the cancer cells. At this time, the cancer cells present a complex of MHC class I antigen and cancer antigen on their cell surface, and this is targeted by the killer T-cells (Cur. Opin. Immunol., 5, 709, 1993; Cur. Opin. Immunol., 5, 719, 1993; Cell, 82, 13, 1995; Immunol. Rev., 146, 167, 1995).

**[0004]** The aforementioned cancer antigen presented by MHC class I antigen on the cancer cells serving as the target cells is thought to be a peptide composed of about 8-12 amino acids formed as a result of antigen protein synthesized within cancer cells being processed by intracellular protease (Cur. Opin. Immunol., 5, 709, 1993; Cur. Opin. Immunol., 5, 719, 1993; Cell, 82, 13, 1995; Immunol. Rev., 146, 167, 1995).

**[0005]** At present, although searches have been conducted for antigen proteins for various cancers, few have been verified to be cancer-specific antigens.

**[0006]** The tumor suppresser gene WT1 of Wilms tumor (WT1 gene) has been isolated from chromosome 11p13 as one of the causative genes of Wilms tumor based on analysis of the WAGR syndrome that occurs as a complication of Wilms tumor, aniridia, urogenital abnormalities, mental retardation and so forth (Gessler, M., et al., Nature, Vol. 343, p. 774-778 (1990)). Its genomic DNA is about 50 kb and is composed of 10 exons, while its cDNA is about 3 kb. The amino acid sequence estimated from cDNA is as shown in Sequence ID No. 1 (Mol. Cell. Biol., 11, 1707, 1991).

**[0007]** The WT1 gene is expressed with high frequency in human leukemia, and when leukemia cells are treated with WT1 antisense oligomer, the growth of the cells is inhibited (Japanese Unexamined Patent Publication No. 9-104627). Thus, WT1 gene is thought to act to promote the growth of leukemia cells. Moreover, WT1 is also highly expressed in solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germinal cell cancer, liver cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical carcinoma and ovarian cancer (Japanese Patent Application No. 9-191635), and the WT1 gene has been demonstrated to be a novel tumor marker in leukemia and solid cancers.

**[0008]** Several cancer-specific antigen peptides consisting of a portion of the WT1 gene expression product are described in WO 00/06602, one particularly promising peptide is designated as $D^b$, and the following amino acid sequence: Cys Met Thr Trp Asn Gln Met Asn Leu (Sequence ID No. 2) (referred to as "WT1 wild peptide" in the present invention) is described therein.

### DISCLOSURE OF THE INVENTION

**[0009]** Thus, an object of the present invention is to provide a peptide that is promising as a cancer vaccine and which has higher activity than previously known cancer-specific antigen peptides.

**[0010]** As a result of earnestly conducting various studies to solve the above problems, the inventors of the present invention found that a peptide (referred to as "WT1 modified peptide") having an amino acid sequence in which the second amino acid Met of the aforementioned known amino acid sequence (Sequence ID No. 2) is changed to Tyr, namely Cys Tyr Thr Trp Asn Gln Met Asn Leu (Sequence ID No. 3), has higher activity, thereby leading to completion of the present invention.

**[0011]** Thus, the present invention provides a peptide (WT1 modified peptide) consisting of 9-30 amino acids and

comprising the following amino acid sequence: Cys Tyr Thr Trp Asn Gln Met Asn Leu (Sequence ID No. 3). This peptide is preferably a polypeptide consisting of 9-12 amino acids and comprising the amino acid sequence indicated in Sequence ID No. 3 and, more preferably, a peptide consisting of the amino acid sequence indicated in Sequence ID No. 3.

**[0012]** Moreover, the present invention provides a cancer vaccine having for its active ingredient the aforementioned WT1 modified peptide.

**[0013]** Moreover, the present invention also provides a DNA vaccine against cancer having for its active ingredient DNA coding for the aforementioned peptide.

**[0014]** In addition, the present invention provides antigen-presenting cells on which presented a complex of HLA antigen (MHC class I antigen) and the aforementioned peptide.

**[0015]** Moreover, the present invention also provides cytotoxic T-cells that recognize a complex of HLA antigen and the aforementioned peptide.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0016]**

Fig. 1 is a graph showing the cell killing effects (specific cytolytic activity) on C1R2402 target cells (T), either pulsed or not pulsed with peptide, by effecter cells (E) stimulated with WT1 wild peptide (Sequence ID No. 2) or the WT1 modified peptide of the present invention (Sequence ID No. 3). In the graph, the black circles indicate the cytolytic effect on C1R2402 target cells pulsed with wild peptide by effecter cells stimulated with WT1 modified peptide, the black squares indicate the cytolytic effect on C1R2404 target cells pulsed with wild peptide by effecter cells stimulated with WT1 wild peptide, the white circles indicate the cytolytic effect on C1R2402 target cells not pulsed with wild peptide by effecter cells stimulated with WT1 modified peptide, and the white squares indicate the cytolytic effect on C1R2402 target cells not pulsed with wild peptide by effecter cells stimulated with WT1 wild peptide.

Fig. 2 is a graph showing the cytolytic activity on acute myelocytic leukemia cells endogenously expressing WT1 antigen or on acute myelocytic leukemia cells not expressing WT1 antigen by effecter cells stimulated with WT1 wild peptide or the WT1 modified peptide of the present invention.

Fig. 3 is a graph showing the cell killing effects (specific cytolytic activity) on C1R2402 target cells, either pulsed or not pulsed with peptide, by effecter cells stimulated with WT1 wild peptide or the WT1 modified peptide of the present invention. In the graph, the black circles indicate the cytolytic effect on C1R2402 cells pulsed with wild peptide by effecter cells stimulated with WT1 modified peptide, the black squares indicate the cytolytic effect on C1R2402 target cells pulsed with wild peptide by effecter cells stimulated with WT1 wild peptide, the white circles indicate the cytolytic effect on C1R2402 target cells not pulsed with wild peptide by effecter cells stimulated with WT1 modified peptide, and the white squares indicate the cytolytic effect on C1R2402 target cells not pulsed with wild peptide by effecter cells stimulated with WT1 wild peptide.

Fig. 4 is a graph showing the cytolytic activity on lung cancer cell lines endogenously expressing WT1 or not expressing WT1 by effecter cells stimulated with WT1 wild peptide or the WT1 modified peptide of the present invention.

Fig. 5 is a graph showing the inhibitory effects of anti-HLA class I antibody, anti-HLA class II antibody and anti-CD8 antibody on the cell killing effects (specific cytolytic activity) on C1R2402 target cells pulsed with wild peptide by effecter cells stimulated by WT1 wild peptide or the WT1 modified peptide of the present invention.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

**[0017]** The peptide of the present invention is a peptide consisting of 9-30 amino acids that comprises the amino acid sequence consisting of the 9 amino acids shown in Sequence ID No. 3. Moreover, from the viewpoint of being presented by binding to HLA antigen, the peptide is preferably a peptide consisting of 9-12 amino acids that comprises the amino acid sequence shown in Sequence ID No. 3 and, more preferably, is a peptide having rules (motifs) in the sequence of antigen peptide presented by binding to HLA antigen at that time (J. Immunol., 152, p. 3913, 1994; Immunogenetics, 41, p. 178, 1995; J. Immunol., 155, p. 4307, 1994; J. Immunol., 155, p. 4749, 1995). Moreover, the peptide is most preferably a peptide consisting of an amino acid sequence of the 9 amino acids shown in Sequence ID No. 3.

**[0018]** Furthermore, the aforementioned "peptide comprising the amino acid sequence shown in Sequence ID No. 3" is specifically, for example, a peptide comprising the amino acid sequence shown in Sequence ID No. 3 and extending in the direction of the N-terminal and/or the direction of the C-terminal from the applicable position on WT1 (Sequence ID No. 1) (position nos. 235-243) or from the corresponding position on human WT1 (NCBI Database Accession No. XP012009), that has activity as a cancer antigen peptide.

**[0019]** An example of a method for measuring the activity of the cancer antigen peptide of the present invention is

the method described in J. Immunol., 154, p. 2257, 1995. The following provides an explanation of an outline of this method using the case of the type of HLA being HLA-A24 as an example. First, peripheral blood lymphocytes are isolated from a person positive for HLA-A24 antigen. Next, by stimulating the peripheral blood lymphocytes by adding the peptide of the present invention in vitro, CTL (cytotoxic T-cells) are induced that specifically recognize the complex of the peptide of the present invention and HLA-A24 presented by the antigen-presenting cells.

**[0020]** This induction of CTL can be investigated by, for example, measuring the amounts of various cytokines (e. g., IFN-γ) produced by the CTL by reacting with the complex of antigen peptide and HLA-A24. In addition, induction of CTL can also be investigated by a method in which the cytotoxicity of the CTL is measured with respect to antigen peptide-presenting cells labeled with $^{51}Cr$ or Europium ($^{51}Cr$ Release Assay, Int. J. Cancer, 58, p. 317, 1994; Europium Release Assay, J. Immunol., 154, p. 3991, 1995). Moreover, induction of CTL can also be investigated by referring to the examples described later.

**[0021]** The present invention also relates to a cancer vaccine that has the aforementioned antigen as its active ingredient. This vaccine can be used for the prevention or treatment of cancers of the blood such as leukemia, myelodysplastic syndrome, multiple myeloma and malignant lymphoma, as well as solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germinal cell cancer, liver cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical carcinoma and ovarian cancer. In particular, this vaccine can be applied to patients positive for HLA-A24. This vaccine can be administered orally or parenterally by for example, intraperitoneal, subcutaneous, intracutaneous, intramuscular, intravenous or intranasal administration.

**[0022]** Moreover, administration of the vaccine of the present invention can also be carried out by a method in which monocytes are collected from the peripheral blood of a patient, dendritic cells are extracted from the monocytes, the dendritic cells are pulsed with the peptide of the present invention and then returned to the patient by subcutaneous administration and so forth.

**[0023]** This method is referred to as cytotherapy or dendritic cell (DC) therapy, and the section entitled "Antigen-Presenting Cells" described later should be referred to for further details.

**[0024]** The vaccine, in addition to the peptide administered as the aforementioned active ingredient, may also contain pharmaceutically allowable carriers such as a suitable adjuvant (Clin-Microbiol. Rev., 7, 277-289, 1994), examples of which include a mineral gel like aluminum hydroxide, a surfactant like phosphorous lecithin and a pluronic polyole, a polyanion, a peptide and an oily emulsion. Alternatively, the vaccine may contain other aggregates mixed into liposomes or blended into polysaccharide or the vaccine. The dosage is typically 0.1 μg/kg to 1 mg/kg per day.

**[0025]** In the present invention, DNA that codes the aforementioned polypeptide vaccine can also be used as a vaccine (DNA vaccine). Namely, after inserting nucleic acids, and preferably DNA, that contain nucleic acids that encode the WT1 modified peptide of the present invention into a suitable vector, and preferably an expression vector, cancer immunity can be imparted by administering the vector to an animal. WO 00/6602 or J. Immunol., 160, p. 1717, 1998 and so forth should be referred to for the specific technique used for this DNA vaccine.

**[0026]** In addition, the present invention relates to antigen-presenting cells on which a complex of HLA antigen and the aforementioned peptide is presented. In this example, although potent cell killing activity is observed due to stimulation with the peptide of the present invention, this is the result of the presence of antigen-presenting cells, on which a complex of the peptide of the present invention and HLA antigen (HLA-A24 antigen) is presented, within peripheral blood monocytes, and of the induction of CTL (cytotoxic T-cells) that specifically recognize these antigen-presenting cells. These antigen-presenting cells on which a complex of HLA antigen and the peptide of the present invention is presented are used effectively in cytotherapy (DC therapy) as described below.

**[0027]** The antigen-presenting cells used in cytotherapy are produced by isolating cells having the ability to present antigen from tumor patients, pulsing these cells with peptide of the present invention outside the body, and causing a complex of HLA antigen and the peptide of the present invention to be presented on the surface of the cells. Here, although there are no particular restrictions on the "cells having the ability to present antigen" provided they are cells that express HLA antigen capable of presenting the peptide of the present invention on the surface of the cells, dendritic cells are preferable since they are considered to have high antigen-presenting ability.

**[0028]** In addition, the peptide of the present invention that is used to pulse the aforementioned cells having the ability to present antigen may not only be in the form of a peptide, but rather may also be in the form of DNA or RNA that encodes said peptide.

**[0029]** A specific method for preparing the antigen-presenting cells of the present invention can be referred to in, for example, Cancer Immunol. Immunother., 46, 82, 1998, J. Immunol., 158, p. 1796, 1997, and Cancer Res., 59, p. 1184, 1999. In the case of using dendritic cells, lymphocytes are isolated from the peripheral blood of a tumor patients using the Fycoll method, and after subsequently removing the non-adhered cells, dendritic cells are derived from the adhered cells by culturing in the presence of GM-CSF and IL-4, after which the antigen-presenting cells of the present invention can be prepared by pulsing the dendritic cells by culturing with the peptide of the present invention.

**[0030]** In addition, in the case of preparing the antigen-presenting cells of the present invention by inserting DNA or RNA encoding the peptide of the present invention into the aforementioned cells having the ability to present antigen,

insertion can be carried out by referring to, for example, Cancer Res., 56, p. 5672, 1996 or J. Immunol., 161, p. 5607, 1998 in the case of DNA, or by referring to J. Exp. Med., 184, p. 465, 1996 in the case of RNA.

**[0031]** These antigen-presenting cells can be used as the active ingredient of a tumor therapeutic agent. At that time, in order to maintain the stability of the antigen-presenting cells, the treatment agent preferably comprises physiological saline, phosphate-buffered saline (PBS) or medium and so forth. Examples of administration methods include intravenous administration, subcutaneous administration and intracutaneous administration.

**[0032]** Moreover, the present invention also relates to cytotoxic T-cells (CTL) that recognize a complex of HLA antigen and the aforementioned peptide. The CTL of the present invention can be effectively used in the adoptive immunotherapy described below.

**[0033]** Namely, in the case of melanoma, adoptive immunotherapy has been recognized to be therapeutically effective by culturing a large number of the patient's T-cells that have invaded the tumor in vitro, and then returning them to the patient (J. Natl. Cancer Inst., 86, 1159, 1994). In addition, in the case of mouse melanoma, inhibition of metastasis has been observed by stimulating spleen cells in vitro with tumor antigen peptide TRP-2, allowing specific CTL to proliferate in the tumor antigen peptide, and then administering said CTL to melanoma-transplanted mice (J. Exp. Med., 185, 453, 1997). This is based on the result of allowing CTL to proliferate in vitro that specifically recognize a complex of the HLA antigen of antigen-presenting cells and tumor antigen peptide. Thus, a treatment method in which patient peripheral blood lymphocytes are stimulated in vitro using the peptide of the present invention to increase tumor-specific CTL followed by returning these cells to the patient is thought to be useful.

**[0034]** In this manner, the CTL of the present invention can be used as the active ingredient of a tumor therapeutic agent. At that time, in order to maintain the stability of the CTL, the therapeutic agent preferably comprises physiological saline, phosphate-buffered saline (PBS) or medium and so forth. Examples of administration methods include intravenous administration, subcutaneous administration and intracutaneous administration.

**[0035]** The following examples serve to clarify the usefulness of the peptide of the present invention as a cancer antigen and cancer vaccine.

Example 1

**[0036]** Peripheral blood mononuclear cells were isolated from HLA-A*2402-positive donors and distributed among the wells of a 24-well plate at 2 x $10^6$ cells/well followed by the addition of WT1 wild peptide or WT1 modified peptide to a concentration of 20 μM and culturing for 1 week. The medium used at this time consisted of 45% RPMI, 45% AIV, 10% FCS 1x non-essential amino acids and SM/PCG. Following the aforementioned culturing, the cells were adjusted to 2 x $10^6$ cells/well and used as responder cells.

**[0037]** On the other hand, other peripheral blood mononuclear cells were similarly isolated from the same HLA-A*2402-positive donors and then peptide-pulsed by culturing for 4 days with one of the aforementioned peptides at 20 μM. After then irradiating at 30 Gy, the cells were adjusted to 4 x $10^6$ cells/well and used as stimulator cells.

**[0038]** The responder cells and stimulator cells prepared in the manner described above were then mixed and then cultured for 1 week following the addition of IL-2 at 50 U/ml. As a result, the status of the resulting cells were as shown in the following table.

Table 1

| Peptide | No. of cells | CD4 | CD8 |
|---|---|---|---|
| WT1 wild peptide | 2.4 x $10^6$/well | 5% | 35% |
| WT1 modified peptide | 3.0 x $10^6$/well | 18% | 38% |

**[0039]** Next, a killing assay was carried out in accordance with $^{51}Cr$ release method (J. Immunol., 164, 1873, 2000). C1R2402 cells and C1R2402 cells pulsed with the aforementioned peptides were used for the target cells. Cells stimulated by WT1 wild peptide or WT1 modified peptide as previously described (effector cells (E)) were then allowed to act on each of these target cells (T) at an E:T ratio of 1, 5 of 20, followed by measurement of cell lysis. Those results are shown in Fig. 1. As is clear from this graph, cells stimulated with WT1 modified peptide exhibited a more potent cell killing activity than cells stimulated with WT1 wild peptide.

Example 2

**[0040]** The cell killing activity of effector cells stimulated with WT1 wild peptide or WT1 modified peptide on leukemia cells that endogenously express WT1 antigen was tested according to the $^{51}Cr$ release method. WT1+/A*2402+ cells (leukemia cells from AML patient #1), WT1-/A*2402+ cells (leukemia cells from AML patient #2), WT1+/A*2402- cells

(leukemia cells from AML patient #3) and WT1-/A*2402- cells (leukemia cells from AML patient #4) were used for the target cells.

**[0041]** The effector cells (E) prepared in Example 1 and the aforementioned target cells (T) were mixed at an E:T ratio of 20:1 and cultured for 4 hours followed by measurement of the degree of cell lysis. Those results are shown in Fig. 2.

**[0042]** As is clear from this graph, although both the cells stimulated with WT1 wild peptide or WT1 modified peptide demonstrated cytotoxic activity on the WT1+/A*2402 cells, the level of that activity was higher for the WT1 modified peptide.

Example 3

**[0043]** The same experiment as Example 1 was carried out using effector cells prepared from peripheral blood mononuclear cells of different healthy donors positive for HLA-A*2402. Those results are shown in Fig. 3.

**[0044]** As is clear from this graph, similar to Example 1, cells stimulated with WT1 modified peptide exhibited a more potent cytotoxic activity than cells stimulated with WT1 wild peptide.

Example 4

**[0045]** The cytotoxic activity of effector cells stimulated with WT1 wild peptide or WT1 modified peptide was tested on a cancer cell line associated with lung cancer that endogenously expresses WT1 antigen (target cells) using the $^{51}Cr$ release method. RERF-LCAI (WT1+/A*2402+), LC1sq (WT1+/A*2402+), 11-18 (WT1-/A*2402+) and LK87 (WT1+/A*2402-) cells were used for the target cells.

**[0046]** Effector cells (E) prepared in the same manner as Example 1 and the aforementioned target cells (T) labeled with $^{51}Cr$ were cultured for 4 hours at an E:T ratio of 20:1 in the same manner as Example 2 followed by measurement of the degree of cell lysis. Those results are shown in Fig. 4.

**[0047]** As is clear from this graph, although both the cells stimulated with WT1 wild peptide or WT1 modified peptide demonstrated cytotoxic activity only on the WT1+/A*2402+ cells, the level of that activity was higher for WT1 modified peptide.

Example 5

**[0048]** Effector cells stimulated with WT1 wild peptide or WT1 modified peptide were confirmed to be CD8-positive killer cells that bind to HLA class I by a blocking assay using antibody. The antibodies used consisted of anti-HLA class I antibody, anti-HLA class II antibody and anti-CD8 antibody. Effector cells (E) prepared in the same manner as Example 1 and target cells (T) in the form of C1R2402 cells or C1R2402 cells pulsed with WT1 wild peptide, both labeled with $^{51}Cr$, were mixed with antibody at an E:T ratio of 20:1 and then cultured for 4 hours followed by measurement of the degree of cell lysis according to the $^{51}Cr$ release method. Those results are shown in Fig. 5.

**[0049]** As is clear from this graph, cytotoxic activity was blocked by anti-HLA class I antibody and anti-CD8 antibody for both the cells stimulated with WT1 wild peptide or WT1 modified peptide, indicating that the cells that exhibit cytotoxic activity are CD8-positive killer cells that bind to HLA class I.

Example 6

**[0050]** The binding affinity of WT1 modified peptide and WT1 wild peptide to HLA-A*2402 was investigated. After treating C1RA2402 cells for 1 minute with a buffer solution (131 mM citric acid, 66 mM sodium phosphate, 290 m osmol, pH 3.3), the cells were neutralized by adding DMEM medium comprising 0.5% bovine serum albumin. After washing the cells with the medium, they were suspended at a concentration of $2 \times 10^6$ cells/ml in DMEM medium containing 200 nM β2-microglobulin (Sigma) and 0.5% bovine serum albumin. 15 μl of the cell suspension were mixed with 50 μl of the medium comprising various concentrations of WT1 peptide followed by incubating for 4 hours at room temperature. After washing the cells, they were stained with monoclonal antibody to HLA-A24 labeled with FITC (clone name: 7A12), and the amount of HLA-A24 expressed was analyzed with a flow cytometer FACS system. A similar procedure was performed on the antigen peptide of melanoma antigen pmel 15, which has been reported to bind to HLA-A*2402 (Ala Tyr Gly Leu Asp Phe Tyr Ile Leu) (Sequence ID No. 4) (J. Immunol., 154, 5994, 1995), and using this as a standard, the dissociation constants (Kd) of the WT1 peptides were calculated according to the method described in the literature (Immunogenetics, 51, 816, 2000). These results are shown in Table 2.

Table 2

| Peptide | Dissociation constant Kd (M) |
|---|---|
| WT1 wild peptide | $1.82 \times 10^{-5}$ |
| WT1 modified peptide | $6.40 \times 10^{-7}$ |

**[0051]** As is clear from this table, WT1 modified peptide demonstrated stronger binding affinity for HLA-A*2402 than the WT1 wild peptide.

**[0052]** On the basis of the aforementioned results, the peptide of the present invention was proven to unquestionably function as a cancer antigen, and cause the induction and proliferation of killer T-cells (cancer cell cytotoxic T-cells) against cancer cells. Thus, the cancer antigen peptide of the present invention is useful as a cancer vaccine against leukemia and solid cancers accompanying an increased expression of the WT1 gene.

SEQUENCE LISTING

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     50                  55                  60
Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro Ser Trp Gly Gly
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## Claims

1. A cancer antigen peptide having for its active ingredient a peptide consisting of 9-30 amino acids and comprising the following amino acid sequence: Cys Tyr Thr Trp Asn Gln Met Asn Leu (Sequence ID No. 3).

2. A cancer antigen peptide according to claim 1 that is consisted of 9-12 amino acids and comprises the amino acid sequence indicated in Sequence ID No. 3.

3. A cancer antigen peptide according to claim 1 that is consisted of the amino acid sequence indicated in Sequence ID No. 3.

4. A cancer vaccine having for its active ingredient a peptide according to any of claims 1 through 3.

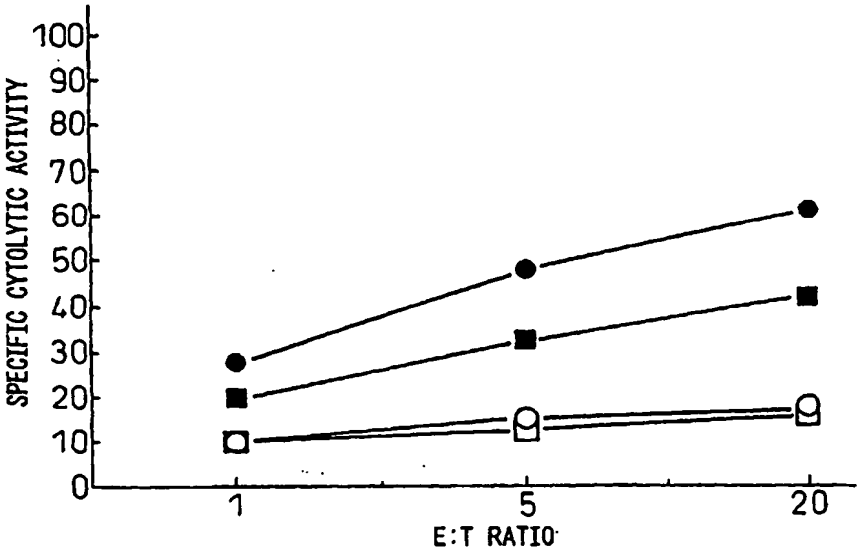
5. A DNA vaccine against cancer having for its active ingredient DNA that codes for a peptide according to any of

claims 1 through 3.

6. Antigen-presenting cells on which are presented a complex of HLA antigen and a peptide according to any of claims 1 through 3.

7. Cytotoxic T-cells that recognize a complex of HLA antigen and a peptide according to any of claims 1 through 3.

Fig.1




—●— [EFFECTER CELLS STIMULATED WITH WT1 MODIFIED PEPTIDE
C1R2402 TARGET CELLS PULSED WITH WILD PEPTIDE

—■— [EFFECTER CELLS STIMULATED WITH WT1 WILD PEPTIDE
C1R2402 TARGET CELLS PULSED WITH WILD PEPTIDE

—○— [EFFECTER CELLS STIMULATED WITH WT1 MODIFIED PEPTIDE
C1R2402 TARGET CELLS NOT PULSED WITH WILD PEPTIDE

—□— [EFFECTER CELLS STIMULATED WITH WT1 WILD PEPTIDE
C1R2402 TARGET CELLS NOT PULSED WITH WILD PEPTIDE

Fig.2

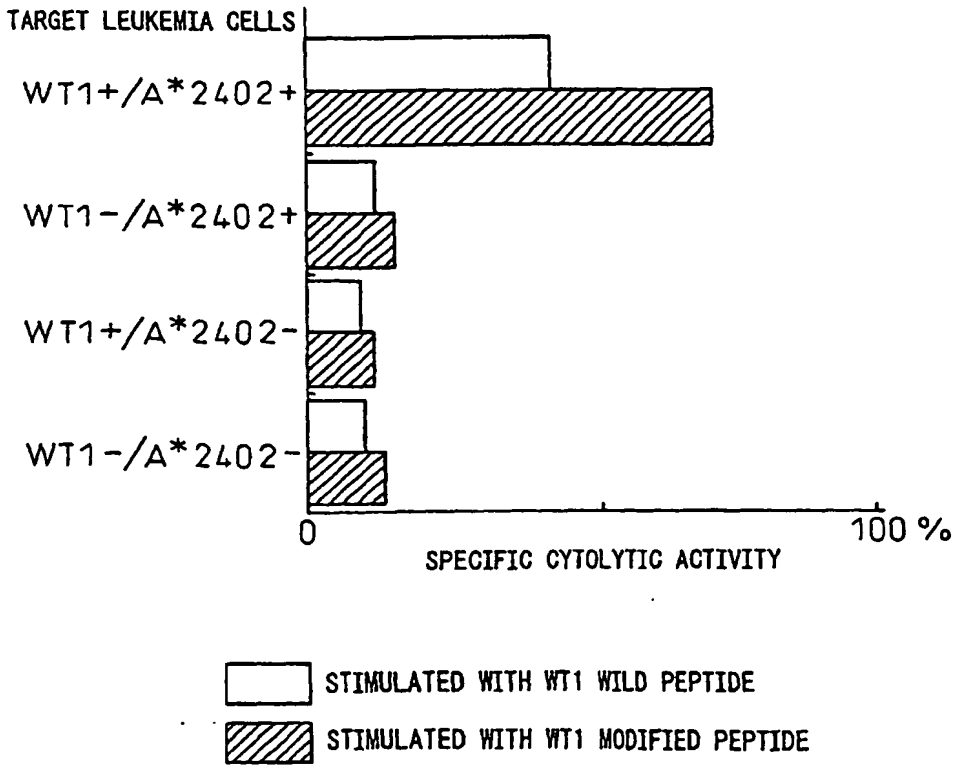
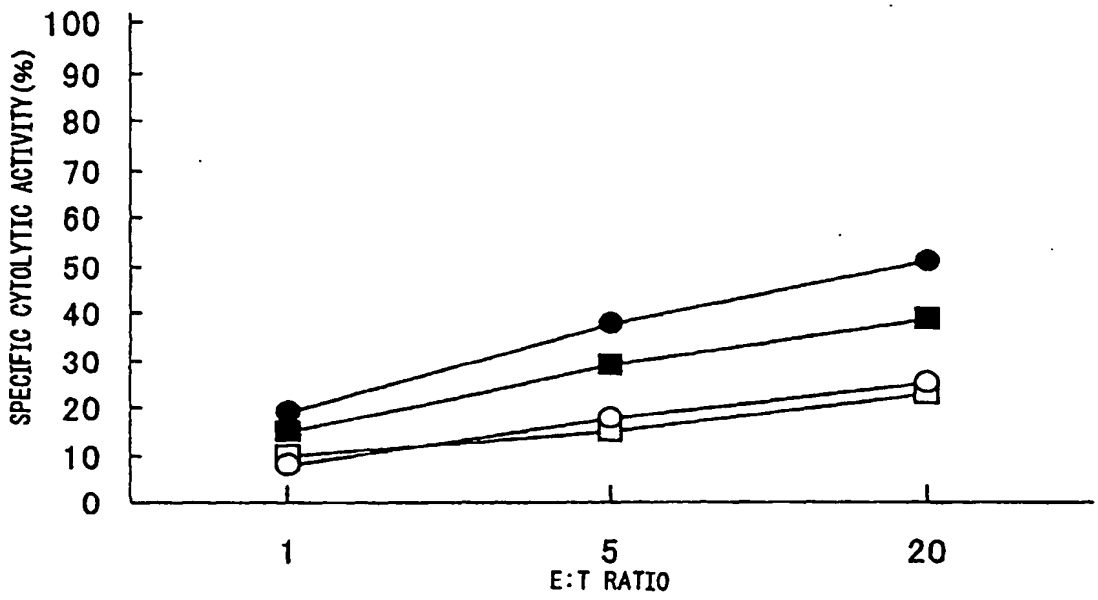

TARGET LEUKEMIA CELLS
WT1+/A*2402+
WT1-/A*2402+
WT1+/A*2402-
WT1-/A*2402-
0
100 %
SPECIFIC CYTOLYTIC ACTIVITY
STIMULATED WITH WT1 WILD PEPTIDE
STIMULATED WITH WT1 MODIFIED PEPTIDE

Fig.3




EFFECTER CELLS STIMULATED WITH WT1 MODIFIED PEPTIDE
C1R2402 TARGET CELLS PULSED WITH WILD PEPTIDE

EFFECTER CELLS STIMULATED WITH WT1 WILD PEPTIDE
C1R2402 TARGET CELLS PULSED WITH WILD PEPTIDE

EFFECTER CELLS STIMULATED WITH WT1 MODIFIED PEPTIDE
C1R2402 TARGET CELLS NOT PULSED WITH WILD PEPTIDE

EFFECTER CELLS STIMULATED WITH WT1 WILD PEPTIDE
C1R2402 TARGET CELLS NOT PULSED WITH WILD PEPTIDE

Fig.4

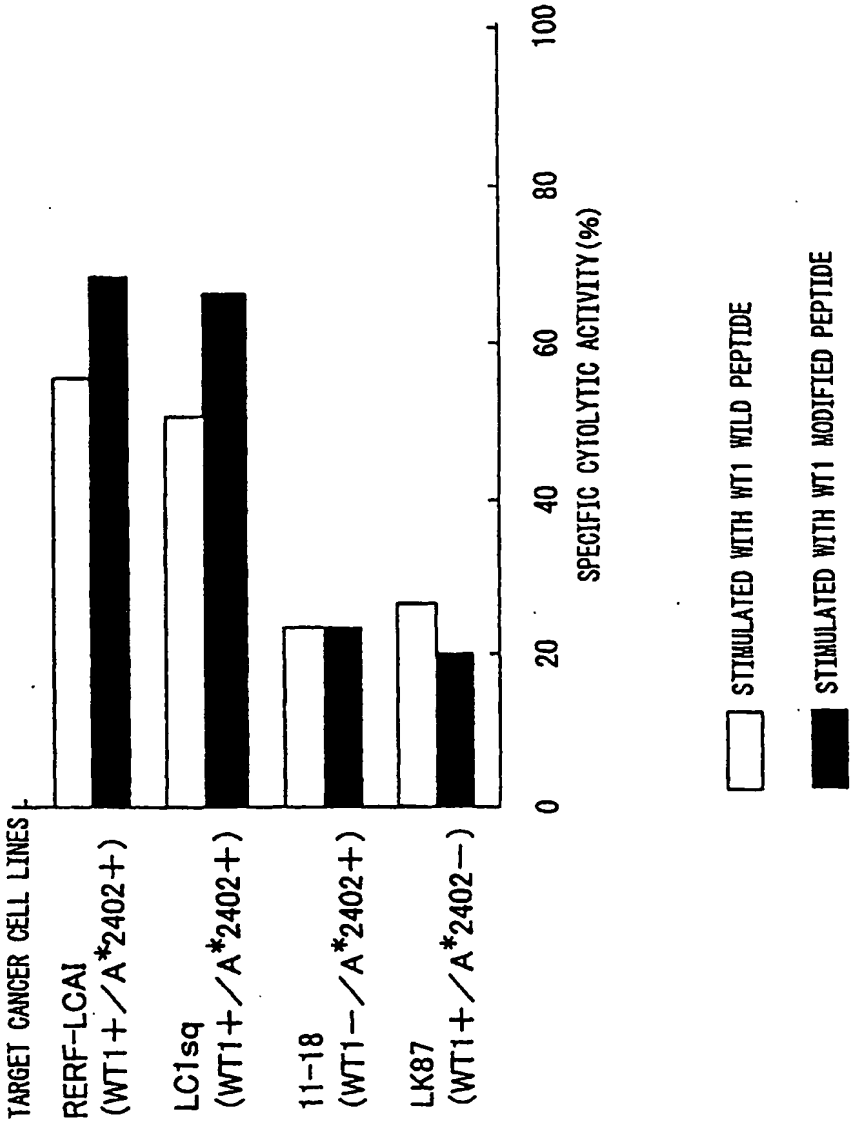
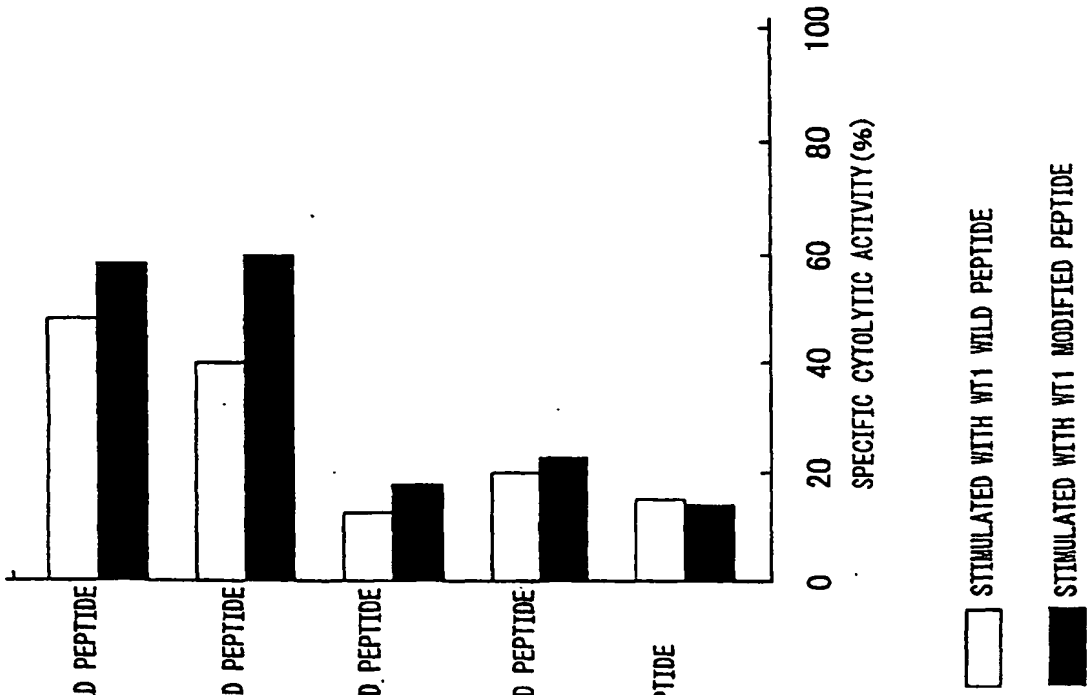

TARGET CANCER CELL LINES
RERF-LCAI
(WT1+/A*2402+)
LC1sq
(WT1+/A*2402+)
11-18
(WT1-/A*2402+)
LK87
(WT1+/A*2402-)
0
20
40
60
80
100
SPECIFIC CYTOLYTIC ACTIVITY(%)
STIMULATED WITH WT1 WILD PEPTIDE
STIMULATED WITH WT1 MODIFIED PEPTIDE

Fig.5


TARGET CELLS PULSED WITH WT1 WILD PEPTIDE ANTIBODY NOT ADDED
TARGET CELLS PULSED WITH WT1 WILD PEPTIDE ANTI-HLA CLASS II ANTIBODY ADDED
TARGET CELLS PULSED WITH WT1 WILD PEPTIDE ANTI-HLA CLASS I ANTIBODY ADDED
TARGET CELLS PULSED WITH WT1 WILD PEPTIDE ANTI-CD8 ANTIBODY ADDED
TARGET CELLS NOT PULSED WITH PEPTIDE ANTIBODY NOT ADDED
0
20
40
60
80
100
SPECIFIC CYTOLYTIC ACTIVITY(%)
STIMULATED WITH WT1 WILD PEPTIDE
STIMULATED WITH WT1 MODIFIED PEPTIDE

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/02794

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C07K14/82, A61K38/00, A61K39/00, A61P35/00, C07K7/04, C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C07K14/82, C07K7/04, C12N5/06, C12N15/09

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
REGISTRY(STN), CA(STN), MEDLINE(STN), WPI(DIALOG), BIOSIS(DIALOG), JICST FILE(JOIS), GenBank/EMBL/DDBJ/GeneSeq, SwissProt/PIR/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>A | WO 00/26249 A1 (Imperial College Innovations Ltd.),<br>11 May, 2000 (11.05.00),<br>Claims<br>& AU 9964797 A & EP 1127068 A1 | 7<br>1-6 |
| X<br>A | WO 00/18795 A2 (Corixa Corp.),<br>06 April, 2000 (06.04.00),<br>Claims<br>& AU 9964078 A & EP 1117687 A2<br>& BR 9914116 A & CN 1336935 A<br>& NO 200101613 A & KR 2001085861 A<br>& HU 200103598 A2 | 7<br>1-6 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 June, 2002 (14.06.02) | 25 June, 2002 (25.06.02) |
| Name and mailing address of the ISA/<br>Japanese Patent Office<br><br>Facsimile No. | Authorized officer<br><br>Telephone No. |

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## INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/02794

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 00/06602 A1 (Haruo SUGIYAMA), 10 February, 2000 (10.02.00), Full text<br>& AU 9949321 A & BR 9912663 A<br>& EP 1103564 A1 & JP 2000-562398 A<br>& CN 1314916 A & KR 2001072112 A | 1-7 |
| A | OKA, Y. et al., Cancer Immunotherapy Targeting Wilms' Tumor Gene WT1 Product, J.Immunol. (2000) Vol.164, pages 1873 to 1880 | 1-7 |
| A | Keiko TADOKORO, "Gan Yokusei Idenshi WT1 no Kino Hatsugen", Gendai Kagaku extra issue 33 "Gan Idenshi Kenkyu no Tenbo II" (1997), pages 92 to 98 | 1-7 |
| A | WO 96/38176 A1 (Chuzo KISHIMOTO), 05 December, 1996 (05.12.96), Full text<br>& AU 9657796 A & JP 9-104629 A<br>& EP 841068 A1 & US 6034235 A | 1-7 |

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